**Supplementary Data**

**Methods**

*Method development*

Chromatogram of stigmasterol was developed in the selected mobile phase after trying various combinations of solvents. The best resolution was observed in the mobile phase containing n-hexane and ethyl acetate in the ratio of 8:2 (v/v). The identical mobile phase was used to separate the different phytoconstituents present in APPeE. The optimized saturation time was observed as 20min. The densitometry analysis was performed at absorption maxima of wave length 540 nm in absorbance mode.

*Method validation*

The planned technique was validated as per the ICH guidelines (ICH guideline, 2005). For determining the linearity range of standard stigmasterol (100µg/mL), a series of spots of different volumes (1µL-12µL) were applied so as to get 100-1200ng quantity of stigmasterol per band. Graph was plotted between concentration and peak area for linearity. Linearity data was statistically treated using least square linear regression analysis. Accuracy was determined by standard addition method. The standard solution was spiked with the extra 0, 50, 100 and 150% and was reanalyzed in six replicates by the planned technique. The % recovery and percent relative standard deviation (% RSD) were calculated. Precision (inter and intraday) of the planned technique was assessed by performing replicate analyses (n=6) at 400, 600 and 800ng/spot of stigmasterol. Inter-day precision was determined by repeating the intra-day assay on three different days. Robustness was studied in triplicate at 400ng/ band by making small changes to mobile phase composition, volume and duration of saturation. The results were studied in terms of SD and % RSD of peak areas. Mobile phases prepared from n-hexane: ethyl acetate in different proportions (8: 2, v/v; 7.8: 2.2, v/v; 8.2: 1.8, v/v) were used for chromatography. Mobile phase volume and duration of saturation investigated were 20 ± 2 mL (18, 20, and 22mL) and 20 ± 10 min (10, 20 and 30min), respectively. The plates were activated at 110°C for 30 minutes before chromatography.

*Assay of stigmasterol*

Different concentrations of stigmasterol and test sample were spotted on HPTLC plates. The percentage of stigmasterol present in APPeE was determined by measuring area for the standard and test sample.

**Results**

*HPTLC method development and validation*

The solvents n-hexane and ethyl acetate in the ratio of 8:2, v/v was found as the best mobile phase which furnish a sharp peak of stigmasterol at Rf value of 0.19 ± 0.001 (Supplementary Fig. 1A). The developed method effectively separated the constituents present in the APPeE (Supplementary Fig. 1B) at a wave length of 540 nm in absorbance mode. The extract bands were confirmed by encrusting their spectra with stigmasterol spectral data (Supplementary Fig. 1C). The calibration curve of stigmasterol was found to be linear in the range 100-1200 ng/spot (Supplementary Table 1). The correlation coefficient (r2) for stigmasterol were 0.9959 and found to be highly significant (P<0.001). The linear regression equation was Y= 6.98X + 223.86, where Y is response and X is amount of reference standards. The limit of detection (LOD) and limit of quantification (LOQ) for stigmasterol were found to be 13.82 ng/band and 41.89 ng/band (Supplementary Table 1). The accuracy was calculated by recovery analysis which afforded recovery of 98.75 -99.44% and the different values are listed in Supplementary Table 2. Low values of % RSD (0.97-1.39) indicated good accuracy of the proposed method. The statistical analysis proved that the developed method is reproducible and selective. Intra-day and inter-day precision of the assay of stigmasterol at three different concentration levels (400, 600 and 800 ng/band) were expressed as RSD (%) in Supplementary Table 3. % RSD was in the range 1.17-1.30 & 1.07-1.27, respectively for intra-day and inter-day precision. These low values indicated that the method was precise. Results of robustness are shown in Supplementary Table 4. Low values of % RSD and so proved that the proposed HPTLC method is robust.

*HPTLC analysis of stigmasterol in Aloe perryi petroleum ether extract (APPeE)*

The utility of the proposed method was evaluated by applying this method for the quantification of stigmasterol in APPeE. The quantity of biomarker stigmasterol was found to be 0.238% w/w of dried weight of *APPeE*.



**Supplementary Fig. 1.** Quantification of stigmasterol in *Aloe perryi* petroleum ether extract (APPeE) by HPTLC using hexane: ethyl acetate (8:2, v/v) as mobile phase at λmax = 540 nm. (A) Chromatogram of standard stigmasterol (Rf = 0.19) and APPeE (stigmasterol, spot 4, Rf = 0.19); (B) Pictogram of p-anisaldehyde derivatized TLC plate in day light; (C) Spectral comparison of the extract with standard at wavelength of 540 nm.

**Reference**

*International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human use, Harmonised Triplicate Guideline on Validation of Analytical Procedures: Text and Methodology Q2 (R1), Complementary Guideline on Methodology incorporated in November 2005 by the ICH Steering Committee, IFPMA, Geneva.*

**Supplementary Table 1:** Rf, Linear regression data for the calibration curve of stigmasterol (n=6)

|  |  |
| --- | --- |
| **Parameters**  | **Stigmasterol** |
| Linearity range (ng/spot) | 100-1200 |
| Regression equation  | Y= 6.98X+ 223.86 |
| Correlation *(r2)* coefficient |  0.9959 |
| Slope ± SD |  6.98 ± 0.03 |
| Intercept ± SD  | 223.86 ± 16.04 |
| Standard error of slope | 0.01 |
| Standard error of intercept  | 7.44 |
| Rf | 0.19 ± 0.001 |
| LOD (ng)LOQ (ng)  | 13.8241.89 |

**Supplementary Table 2:** Recovery as accuracy studies of the proposed HPTLC Method (n=6)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Percent (%) of stigmasterol added to analyte** | **Theoretical concentration of stigmasterol (ng/band)** | **Concentration found (ng/band) ± SD** | **% RSD** | **% Recovery**  |
| 0 | 400 | 397.76 **±** 5.51 | 1.39 | 99.44 |
| 50 | 600 | 592.48 **±** 7.09 | 1.20 | 98.75 |
| 100 | 800 | 792.73 **±** 8.43 | 1.06 | 99.09 |
| 150 | 1000 | 989.93 **±** 9.61 | 0.97 | 98.99 |

**Supplementary Table 3:** Precisionof the proposed HPTLC Method (n=6)

|  |  |
| --- | --- |
| **Conc. of standard added (ng/band)** | **Stigmasterol**  |
| **Intra-day Precision** | **Inter-day Precision** |
| **Average Conc. found ± SD** | **%RSD** | **Average Conc. found ± SD** | **%RSD** |
| 400 | 398.62 ± 5.17 | 1.30 | 396.47 ± 5.03 | 1.27 |
| 600 | 596.59 ± 7.29 | 1.22 | 593.72 ± 6.67 | 1.12 |
| 800 | 793.49 ± 9.43 | 1.17 | 790.62 ± 8.53 | 1.07 |

**Supplementary Table 4:** Robustness of the proposed HPTLC Method (n=6)

|  |  |
| --- | --- |
| **Optimization condition** | **Stigmasterol (400 ng/band)** |
| **SD** | **% RSD** |
| **Mobile phase composition;****(Hexane: Ethyl acetate)** |  |
| (8 : 2) | 5.39 | 1.36 |
| (7.8 : 2.2) | 5.27 | 1.33 |
| (8.2 : 1.8) | 5.19 | 1.31 |
| **Mobile phase volume****(for saturation)** |  |
| (18 mL) | 5.18 | 1.31 |
| (20 mL) | 5.14 | 1.30 |
| (22 mL) | 5.15 | 1.29 |
| **Duration of saturation** |  |
| (10 min) | 5.43 | 1.38 |
| (20 min) | 5.39 | 1.36 |
| (30 min) | 5.31 | 1.35 |